Notochordal cells activate nucleus pulposus cells more strongly after stimulation with serum in 3D cross-species co-cultures

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INTRODUCTION: Notochordal Cells (NC) are shifted back into focus due to their apparent action of activating other disc cells via indirect release of yet unknown factors into the medium (conditioned medium = CM).[1,2] Recent evidence confirms the results from the late 90ies.[3,4] Here, we test porcine (p) NC cultured in 3D and the influence of adding serum or using serum-free medium onto the culture on NC cells and its stimulating effects for subsequent co-culture with primary bovine (b) nucleus pulposus (bNPC) and annulus fibrous cells (bAFC).

METHODS: Primary pNC, bNPC and bAFC were isolated from fresh porcine tails (< 6-12 months age) or bovine tails (~1 yr age), which were obtained from the food chain. All Cells were seeded into 1.2% alginate, each with a density of 4 x 10^6 /mL. NC were then either cultured for 7 days in serum free medium (SFM = Dulbecco's Modified Eagle Medium DMEM supplied with ITS+, 50µg/mL vitamin C and non-essential amino acids) or DMEM + 10% fetal calf serum (FCS). CM was produced from NC collecting 4mL SFM and keeping ~30 beads for 7 days. Then, a co-culture was set-up in SFM for 14 days using indirect cell-cell contact (culture insert, high density pore, 0.4 µm) using a 50:50% ratio[5] of pNC:bNP or bAF, or by addition of CM, respectively. The Glycosaminoglycan per DNA (GAG/DNA) ratio, real-time RT-PCR of IVD relevant genes and cell activity was monitored.

RESULTS: GAG/DNA ratio was slightly increased in hypoxia relative to day 0 and relative to normoxia (Fig. 1). bNPC tended to be more strongly activated in hypoxia but in co-culture under a 50:50 ratio with pNC that were kept for 7 days in DMEM + 10% FCS the GAG/DNA ratio was up-regulated by almost 300% (Fig. 1). CM did not show any stimulating effects on bNPC nor on bAFC. Furthermore, cell activity as measured by resazurin red tended to be increased in pNC even after 14 days post-switch to SFM for co-culture experiments.

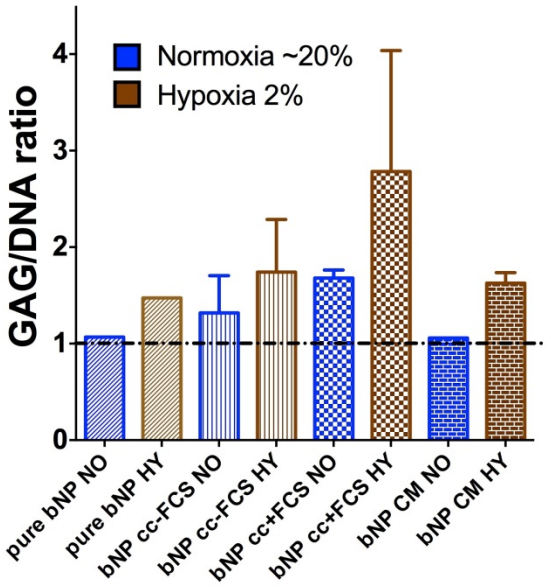

Fig. 1 Barplot of GAG/DNA ratio after 14 days of bNPC in 3D alginate in 1:1 coculture (CC) with porcine NC which were in kept in DMEM +FCS or in SFM (-FCS) for 7 days prior CC. Note the activation of the bNPC in CC with pNC previously cultured in DMEM containing (+) FCS in hypoxia.

DISCUSSION & CONCLUSIONS:

Our results showed a trend that GAG/DNA ratio of the hypoxic condition was higher than in normoxia[6] and that FCS has a stimulating effect to pNC for subsequent co-culture.

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